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Milk mycoflora survey of dairy cows with or without mastitis

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Abstract

Mastitis in cattle is a serious problem which causes considerable economic losses in dairy cattle herds. The aim of this survey was to identify mycoflora in milk of healthy, clinical and subclinical mastitis of lactating Holstein dairy cows. Milk samples of 154 Holstein dairy cows were collected from 10 dairy cattle herds in suburb of Mashhad, Iran. The treatment groups included 104 lactating dairy cows with clinical (38, 25%) and subclinical (66, 43%) mastitis. Fifty (32%) healthy dairy cows were included as control, as well. Different fungi were isolated from cows with clinical (14%), subclinical (18%) mastitis and healthy animals (15%). There were no significant differences between treatment and control groups (P>0.05). It was shown that milk samples of cows with clinical and subclinical mastitis were contaminated with 5 different fungal agents. However, milk samples of healthy cows also were contaminated with 5 kinds of fungal agents. *Yeast* (26%) and *Aspergillus fumigatus* (18%) were the most common isolated agents. It is concluded that fungal infections (mainly *Aspergillus fumigatus*) and yeast can occur in mammary glands of lactating Holstein dairy cows with or without mastitis.

Keywords: Cow, mycoflora, lactating, mastitis

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Introduction

Bovine mastitis is defined as an inflammation of the parenchyma of the mammary gland regardless of the cause. It assumes major economic importance in dairy cattle and maybe one of the most costly diseases in dairy herds. Mastitis results in economic loss for producers by increasing the costs of production and by decreasing productivity which these losses have been divided into reduced milk production (70%), milk discarded after treatment (8%), drugs and veterinary expenses (8%), and culling (14%) (Philpot, 1984, Fang *et al.*, 1993).

Mastitis occurs in either clinical or subclinical forms. Subclinical mastitis is more prevalent than clinical mastitis. However, it is not manifested as visible changes in the mammary glands or in the milk. Therefore, it is not easily recognized by farmers (Radostitis *et al*., 2007). Many researchers have been investigated the causes of clinical and subclinical mastitis especially with bacterial origin, but there are relatively few studies available concerning the survey of fungal agents in lactating cows with clinical and subclinical mastitis which can be common agents in milk of dairy cows. Fungi and **y**easts are common environmental microorganisms. Fungal microorganisms have been isolated from bovine mastitis (Vestweber and Leipold, 1995, Santos and Marin, 2005, Brown *et al*., 2007). They can cause mastitis if a large number of cows lay out of the cubicles (free-stalls) or if the milker washes the teats but does not wipe them dry before applying milking units. However, contaminated water and no sanitization can make the problems more serious. In farms where wet teats are a problem, heavy contamination of teat skin leads to infection in bulk milk (Philpot and Nickerson, 1991Blowey and Edmondson, 1995).

Milk mycoflora of dairy cows with or without mastitis have not received much attention in the past. Therefore, the aim of this study was to evaluate the milk flora status in healthy, clinical and subclinical mastitis of lactating Holstein dairy cows.

Materials and methods

One hundred and fifty four milk samples from lactating Holstein dairy cattle without mastitis (healthy) (50, 32%) or clinical (38, 25%) and subclinical mastitis (66, 43%) were examined in 10 industrial dairy cattle herds in suburb of Mashhad, Iran. Mashhad Suburb in Khorasan Razavi province is a major producer of livestock and dairy production in North-east of Iran.

The samples were determined by taking true randomly using a lottery mechanism in the dairy herds (Thrust field, 2005).The average annual milk production in these herds ranged from 6500 to 8400 Kg of milk per cow. Cows with or without clinical mastitis were determined as clinical symptoms. Cows with or without subclinical mastitis were detected using a California Mastitis Test (CMT) (Radostitis *et al*., 2007). Cows were not taken antibiotic(s) before sampling. Before sampling, the teats were washed, cleaned and disinfected using alcohol 95%, respectively. The first three stripping of milk were discarded. Milk samples (10 ml) were collected from each quarter in sterile tubes and they were transferred to the laboratory on cold condition. They were inoculated in Sabouraud Dextrose Agar (SDA) (Difco Laboratories, USA) containing Chloramphenicol /and SDA with chloramphenicol & cycloheximide at 28℃ for 3 weeks. Chloramphenicol and cycloheximide were used in the agar media for initial fungal isolation. Duplicate culture was used for every sample. The yeast and molds were identified on the basis of colony characteristics, microscopic morphology, sugar fermentation tests and germ tube formation test. The data were analyzed using the $\chi 2$ statistic method.

Results

Totally, 72 (47%) out of 154 milk samples were positive for fungi isolates in healthy

(15%), clinical (14%) and subclinical (18%) mastitis cows. However, 53% of cultures were negative. Table 1 shows the distribution of fungal isolates in bovine mastitis of dairy cattle herds in suburb of Mashhad, Iran. There were no significant differences among the treatment and control groups (P>0.05). Cows with clinical and subclinical mastitis were infected by 5 different fungal species. The healthy cows were infected by 5 fungal isolates too. *Yeast* (19, 26%) and *Aspergillus fumigatus* (13, 18%) were the most common isolated fungi. The different fungal isolated agents in treatment and control groups are shown in Table 2. The most frequency of fungal isolates was obtained from milk of cows with subclinical mastitis (38%). It was shown that cows with clinical mastitis (14%), subclinical (18%) mastitis and healthy (15%) had fungal agents (Table 1). An occurrence of 8%, 14% and 4% of *yeast* isolated from milk of cows with clinical, subclinical and without mastitis was registered, respectively (Table 2).

Table 1. Distribution of mycoflora in dairy cows with or without mastitis in dairy cattle herds in suburb of Mashhad, Iran.

Fungal infection	Experimental groups			Total (%)
	Control (%)	Treatment		
		Clinical mastitis (%)	Subclinical Mastitis (%)	
+	23 (15)	22 (14)	27(18)	72(47)
-	27(18)	16(10)	39(25)	82 (53)
Total	50(32)	38(25)	66(43)	154

Table 2. Different fungi isolated from lactating cows with or without mastitis in dairy cattle herds in suburb of Mashhad, Iran.

Mycoflora	Experimental groups			Total (%)
	Control (%)	Treatment		
		Clinical mastitis (%)	Subclinical mastitis (%)	
Yeast	3(4)	6 (8)	10(14)	19(26)
Aspergillusfumigatus	5(7)	4(6)	4(6)	13(18)
Aspergillusfumigatus&yeast	4(6)	3 (4)	1(1)	8 (11)
Aspergillusflavus	2(3)	3(4)	3(4)	8(11)
Penicillium	2(3)	1(1)	1(1)	4(6)
Aspergillusniger	1(1)	-	1(1)	2(3)
Mucor&yeast	1(1)	1(1)	-	2(3)
Mucor, Aspergillusfumigatus &yeast	-	1(1)	1(1)	2(3)
Aspergillusfumigatus, Geotrichum &yeast	1(1)	-	1(1)	2(3)
Geotrichum&yeast	-	1(1)	1(1)	2 (3)
Mucor	-	-	1(1)	1(1)
Penicillium& Sterile hyphe	1(1)	-	-	1(1)
Aspergillusflavus&yeast	1(1)	-	-	1(1)
Aspergillus SPP &yeast	-	-	1(1)	1(1)
Aspergillusflavus&Penicillium	-	-	1(1)	1(1)
Aspergillusniger&yeast	1(1)	-	-	1(1)
Aspergillusfumigatus&Mucor	-	-	1(1)	1(1)
Aspergillusflavus&Aspergillusfumigatus	-	1(1)	-	1(1)
Penicillium, Aspergillusfumigatus,				1(1)
Geotrichum&yeast	1(1)	-	-	1(1)
Aspergillusfumigatus, Penicillium,				1(1)
Mucor&yeast	-	1(1)	-	1(1)
Total	23 (32)	22 (31)	27 (38)	72

Discussion

We found that *yeast* was the common opportunistic pathogen. However, *A.fumigatus* was isolated from 18% of samples and the other mycotic agents were isolated from cows with or without mastitis (Table 2). Other fungal agents were also isolated in this study.

The survey of fungal isolation is performed in many countries with rates of 6% in Egypt, 1% in Denmark, 10% in Poland, and 12% in Brazil (Costa *et al*., 1993, Aalbaek *et al*., 1994, Krukowaki *et al*., 2000). However, most of cows are infected by microbial agents.

Prolonged and intensive antibiotic therapy is an important predisposing factor in farm animals especially in aspergillosis (Krukowaki *et al*., 2000, Radostitis *et al*., 2007). It is obvious that some fungi such as, *Yeasts* and *A. fumigatus* can cause mastitis in cattle. The infection is introduced by contaminated infusions or teat cup liners (Nicholls *et al*; 1981). Establishment of the infection is encouraged by damage to the mammary epithelium and stimulated by antibiotic therapy. Fungal mastitis can be associated with the presence of teat lesions (Philpot and Nickerson, 1991). Each fungal infection can arise from a saprophytic organic matter, commonly moldy hay or straw or moist feeds such as beet pulp, corn silage, and wet grains. However, most of these organisms are opportunists with different sources including the skin of the udder, hands of milking man, milking machines, treatment instruments, floor, straw, feed, dust, drug mixtures and sanitation solutions (Richard *et al*., 1980). Fungal infections can be the result of the hematogenous spread from gastrointestinal lesions; especially via the omasum lesions (Jensen *et al*., 1994). This spread may be accelerated by application of antibiotics. Any immunosuppressive conditions such as using corticosteroid therapy, infection, metabolic disorders and stress, may facilitate the establishment of the mycotic infection (Jensen *et al*., 1989). In this study, it was shown that the healthy cows had fungal infection such as: *A.fumigatus*, *A.flavus* and *Penicillium* (Table 2). However, mixed fungal and yeast infections were isolated in cows with or without mastitis (Table 2). On the other hand, they can be isolated as the common agents from any milk sample.

Fungi can produce toxins. Aflatoxin is a toxin which has been found in many spoiled feeds especially cottonseed meal, corn, and moldy breed (Hall *et al*., 1989).Common sources of this toxin are *A.flavus* and *Penicillum*. Because the toxin is excreted in cows' milk, however, it is important for public health. Aflatoxinis is an important consideration in the etiology of human hepatocellular carcinoma (Mclean and Dutton, 1995). This mycotoxin can also be present in the meat from animals eating contaminated food, but the risks to human eating the meat are thought to be slight.

The International Agency for Research on Cancer placed aflatoxins B1 and M1 on the list of human carcinogens supported by a positive association between dietary aflatoxin and liver cell cancer (Carvirani, 2008). However, this mycotoxin can be a hazard for human public health.

It was concluded that the fungal agents (mainly *A.fumigatus* and *A.flavus*) and yeasts were isolated as the mycoflora of milk samples in Holstein dairy cows with or without mastitis. However, bacterial agents are the most source of mastitis in dairy cows and also milk of dairy cows can have fungal agents. We suggest that these isolates can be members of the resident or opportunistic mammary gland disorder processes which can be important as a sanitation hazards.

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بررسی فلور قارچی در شیر گاوهای مبتلا به ورم پستان یا فاقد آن

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چکیده

ورم پستان در گاو شیری مشکل جدی است که باعث بروز خسارات اقتصادی قابل توجهی در گله های گاوهای شیری می‌شود. هدف از این بررسی، مشخص کردن فلور قارچی شیر گاوهای شیری نژاد هلشتاین سالم، مبتلا به ورم پستان بالینی و تحت بالینی بـود. نمونـه‌هـای شیر ۱۵۴ رأس گاو شیری نژاد هلشتاین از ۱۰ گله گاوهای شیری اطراف مشهد –ایران جمع آوری شد. گروه‌های درمـان شـامل ۱۰۴ رأس گاو شیری مبتلا به ورم پستان بالینی (۳۸ رأس، ۲۵٪) و تحت بالینی (۶۶ رأس، ۴۳٪) بود. پنجاه (۳۲٪) رأس گاوسالم به عنوان کنتـرل در نظر گرفته شد. قارچ‌های مختلف از گاوهایی با ورم پستان بالینی (۱۴٪)، تحت بالینی (۱۸٪) و سالم (۱۵٪) جـدا شـد. اخـتلاف معنـی داری بین گروه‌های درمان و شاهد وجود نداشت (P>0.05). مشاهده شد که شیر گاوهای مبتلا به ورم پستان بالینی و تحت بالینی آلوده به ۵ نوع عامل قارچی است. اما شیر گاوهای سالم نیز آلوده به ۵ نوع عامل قارچی بـود. مخمـر (۲۶٪) و آسـپرژیلوس فومیگـاتوس (۱۸٪) بیشـترین عوامل جدا شده بودند. می‌توان نتیجه گرفت که آلودگی های قارچی (عمدتاً آسپرژیلوس) و مخمـری مـی تواننـد در غـدد پسـتان گاوهـای شیری نژاد هلشتاین دارا یا فاقد ورم پستان روی دهد.

واژگان کلیدی: گاو، فلور قارچی، شیری، ورم پستان